

CLAIMS:

1. A new method for the evaluation of biological ligand binding using non-radioisotopic
4 immunologically recognizable hapten-conjugated ligands.
2. The new method of claim 1, wherein said biological ligand is a growth factor or any
other biological factors in the forms of small organic compounds, proteins, oligo
DNA or RNA.
- 8 3. The biological ligand of claim 1, where the growth factor or other biological factors
are required to be conjugated with an immunological reactive hapten such as but not
limited to fluorescein, biotin, rhodamine, etc.
4. A biological ligand of claim 1, wherein said immunologically reactive hapten
12 conjugated biological ligand must retain its biological and binding activity.
5. The new method of claim 1, wherein the said hapten-containing biological ligand
bound to the target surface can be subsequently removed.
6. The new method of claim 5, wherein said hapten-containing biological ligand can be
16 examined by an analytical method such as, but not limited to electrophoresis, to show
the molecular weight of the bound ligand.
7. The new method of claim 6, wherein said electrophoretically separated hapten-
containing biological ligand can be transferred to and bound to a membrane support.
- 20 8. The new method of claim 5, wherein said removed hapten-conjugated ligand can be
applied directly to a membrane using dot or slot-blot methods.

Inventor: Philip Cavanaugh (Phone: 949-654-8979 or 313-538-2587). Title: Method for the Detection and Measurement of Hapten-Conjugated Biological Binding Entities by Western and Dot Blot using Anti-Hapten Antibodies. Text: 28 pages total. Figures: 12 pages total.

9. The new method of claims 7 and 8, wherein said membrane-bound hapten-conjugated ligand is detected using by treatment with an enzyme-conjugated anti-hapten antibody, then a color or light producing substrate reacted upon by the antibody's conjugated enzyme.
10. The new method of claims 7 and 8, wherein said membrane-bound hapten-conjugated ligand is detected by treatment with an anti-hapten antibody, then an enzyme-conjugated antibody to the anti-hapten antibody, then a color or light-producing substrate reacted upon by the second antibody's conjugated enzyme.
11. The new method of claims 7 and 8, wherein the determination of said amount of the ligand bound to the target surface can be achieved by referring to a standard curve made using a series of solutions of known concentrations of the hapten-conjugated ligand and the same system for separation, membrane binding, and detection of the ligand.
12. The new method of claim 1 where specific binding of the hapten-conjugated ligand can be determined by competitive binding with non-labeled ligand followed by the same removal, separation, membrane binding, and detection methods.
13. The new method of claim 1, 11, and 12 where the quantified specific binding of the hapten-conjugated ligand can be used to determine receptor numbers on the binding surface for the ligand.
14. The new method of claim 1, where the incubation of viable cells at their normal environmental temperature with the hapten-conjugated ligand followed by the same

removal, separation, membrane binding, and detection methods can be used to measure cellular ligand internalization.

15. The new method of claim 14 where specific cellular internalization of the hapten-
4 conjugated ligand can be determined by competitive internalization with non-labeled
ligand followed by the same removal, separation, membrane binding, and detection
methods.
16. The new method of claim 1, wherein said fluorescein conjugated biological ligand is
8 the iron binding protein and growth factor transferrin.
17. The new method of claim 1, wherein said fluorescein conjugated transferrin is used
for determination of the degree of specific binding of transferrin to cell surfaces and
determination of cell surface transferrin receptor number.
- 12 18. The new method of claim 1, wherein said fluorescein conjugated transferrin is used
for determination of the degree of the aggressiveness of tumor cells.
19. The tumor cells of claim 15 are, but are not limited to, various breast cancer cells.
20. The new method of claim 1, wherein said fluorescein conjugated transferrin is
16 detected by anti-FITC antibodies made in, but not limited to, rabbit.
21. The new method of claim 1, wherein said anti-FITC antibody quantity is determined
by but not limited to an enzyme such as the horse radish peroxidase (HRP)-
conjugated anti-rabbit (but not limited to rabbit) IgG.
- 20 22. The new method of claim 1, wherein said fluorescein conjugated biological ligand is
Concanavalin A (Con A).

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23. The new method of claim 1, wherein said fluorescein conjugated Con A is used for determination of the cell binding capacity for Con A by cell surface Con A-binding glycoproteins which contain terminal glucose or mannose.
- 4 24. The cells of claim 23 are, but are not limited to, rat MTLn3 mammary adenocarcinoma cells.
25. The new method of claim 1, wherein said fluorescein conjugated biological ligand is Annexin V.
- 8 26. The new method of claim 1, wherein said fluorescein conjugated Annexin V is used for the detection of cell apoptosis.
27. The new method of claim 1, wherein said fluorescein-conjugated Annexin V is reacted with adherent cells *in situ* and thus binding of the annexin V to cells in a natural state is achieved.
- 12 28. The new method of claim 1, wherein said fluorescein conjugated biological ligand is Avidin.
29. The new method of claim 1, wherein said biotin-conjugated biological ligand is a specific PCR product.
- 16 30. The new method of claim 1, wherein said biotin-conjugated PCR products are hybridized with an immobilized specific DNA probe and the non-specific PCR products can be washed away and removed.
- 20 X 31. The hybridized biotin-labeled PCR product of claim 19 can be removed, separated by electrophoresis and blotted onto a membrane support.

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32. The hybridized biotin-labeled PCR product of claim 19 can be removed, and dot or slot-blotted directly to a membrane support.
33. The membrane-bound biotin-labeled PCR products of claim 26 can be detected and quantified by enzyme linked anti-biotin antibodies. The enzyme is, but is not limited to, horse radish peroxidase (HRP). The enzyme is detected by application of a color or light-producing substrate for the enzyme.
34. The membrane-bound biotin-labeled PCR products of claim 26 can be detected and quantified by anti-biotin antibodies, followed by incubation with an enzyme-conjugated antibody against the anti-biotin antibody. The enzyme is, but is not limited to, horse radish peroxidase (HRP). The enzyme is detected by application of a color or light-producing substrate for the enzyme.
35. The new method of claim 1, wherein said fluorescein conjugated biological ligand is Insulin.

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